Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

EXPERIMENTAL PNEUMOCOCCUS MENINGITIS IN RABBITS AND DOGS

GORO IDZUMI

From the McManes Laboratory of Experimental Pathology of the University of Pennsylvania, Philadelphia

Three forms of pneumococcus meningitis may occur in human beings: First, that due to direct infection of the meninges from a primary focus in the ear, mastoid cells, nose and accessory sinuses; second, that arising during the course of pneumococcus pneumonia, and third, that developing spontaneously and without a discoverable primary focus of pneumococcus infection elsewhere in the body. The first form of the disease is usually a localized infection of the cerebral meninges, while the other two types are likely to prove to be generalized infections of the cerebral and spinal meninges; all forms are accompanied by a high mortality, as the majority of patients succumb to the infection. During the course of labor pneumonia delirium may develop, and spinal puncture reveals an increased volume of sterile cerebrospinal fluid; this condition is due to an acute meningeal congestion erroneously designated as "serous meningitis," and is not to be confused with pneumococcus meningitis. Recently mixed meningococcus and pneumococcus meningitis has been described by Netter and others and the mortality apparently is not as high as in pneumococcus meningitis.

How the meninges become infected with pneumococci in those forms of the disease with no discoverable primary focus of infection or with a primary focus situated remotely from the meninges, as in the lungs, pleura and pericardium, is unknown; it is possible that the pneumococci gain access to the blood and localize in the meninges as a metastatic lesion or, having gained access to the upper respiratory passages and particularly to the nasal mucous membrane, find their way to the meninges at the base of the brain in a manner analogous to infection with meningococcus by this route, as described by Flexner.[1] Bull[2] has reported the development of meningitis in dogs receiving

Received for publication Jan. 3, 1920.

---

[1] Jour. Exper. Med., 1907, 9, p. 142.

[2] Jour. Exper. Med., 1916, 24, p. 7.

large doses of virulent pneumococci by intravenous injection whereas in experimental meningococcus meningitis, it would appear, according to the experiments of Austrian,[3] that the intravenous injection of virulent meningococci in normal rabbits is not followed by meningitis unless local injury is produced and conditions disturbed by the withdrawal of spinal fluid with or without the subthecal injection of some bland irritant as normal serum.

According to Amoss and Eberson,[4] meningococcus meningitis in monkeys and rabbits cannot be produced by the intravenous injection of meningococci alone; in their experiments such attempts were fruitless unless spinal puncture was conducted during the bacteremia with the subthecal injection of some irritant, as normal serum, which produced physical injury of the meninges and reduced the local mechanism of defense. In the experiments of Weed, Wegefarth, Ayer and Felton[5] meningitis in cats was produced by injecting cultures of B. mucosus intravenously followed by spinal puncture and the removal of spinal fluid during the bacteremia.

PURPOSE OF INVESTIGATION

Similar experiments in the production of pneumococcus meningitis have not been made. For the production of experimental pneumococcus meningitis Lamar has used monkeys injected subthecally in the lumbar region with virulent cultures. Owing, however, to the relatively high cost of these animals and the difficulty of obtaining them in sufficient numbers for extensive chemotherapeutic studies, Dr. Kolmer requested me to make a series of experiments for the twofold purpose of throwing additional light on the mechanism of infection of the meninges with pneumococci and of determining whether a definite form of pneumococcus meningitis could be produced in dogs and large rabbits by

(1) The intravenous injection of virulent pneumococci.

(2) The intravenous injection of virulent pneumococci combined with spinal puncture and the subthecal injection of sterile, bland irritants.

(3) The subthecal injection of virulent pneumococci in the region of the spinal cord.

---

[3] Bull. Johns Hopkins Hosp., 1918, 29, p. 183.

[4] Jour. Exper. Med., 1919, 29, p. 605.

[5] Jour. Amer. Med. Assn., 1919, 72, p. 190.

The rabbit is known to be highly susceptible to the pneumococcus and doubt was entertained regarding the successful production of meningitis before death was produced by a bacteremia, but according to Lamar's experiments, pneumococcus bacteremia frequently followed the subthecal injection of pneumococci in monkeys, and it was hoped that the dosage for rabbits could be so regulated as to permit the development of meningitis before the rabbits succumbed. This has been found possible in these experiments.

The dog, on the other hand, is known to be highly immune to virulent pneumococci injected intravenously, and our main interest was centered on the possibility of producing a localized infection of the subarachnoid space without generalized infections; as will be shown, considerable difficulty was encountered in the production of pneumococcus meningitis in dogs, the resistance of this animal being unusually high to the effects of virulent pneumococci injected not only intravenously but subthecally as well.

TECHNIC

The experiments were made with three strains of pneumococci; type 1 strain was obtained from Dr. Avery of the Rockefeller Institute, type 2 from Dr. Berta Meine and type 3 from Dr. Kolmer.

The type 2 strain came from the spinal fluid of a child of 14 years suffering with pneumococcus meningitis of spontaneous development and ending fatally; the type 3 strain came from the spinal fluid of an adult of 56 years suffering with meningitis secondary to a pneumococcus infection of the mastoid cells and ending fatally. Naturally most interest centered on the results of experiments with type 2 and type 3 strains and especially type 2, inasmuch as both produced meningitis in human beings.

The virulence of these strains was maintained by animal passage and usually killed mice of 17 to 22 gm. in weight in the following doses of 24-hour blood-broth cultures:

	Minimal Lethal Dose			
Strain	In 18 Hours	In 24-30 Hours	In 48 Hours	In 72 Hours
	c c	c c	c c	c c
Type 1..........	....	0.001	0.0001	0.00001
Type 2..........	0.01	0.001	0.0001	0.00001
Type 3..........	0.1	0.1	0.001	0.0001

Twenty-four hour blood-broth cultures were used, and in order to reduce the volume of fluid to be injected subthecally and to avoid the irritant properties of the broth alone for the meninges, the cultures were pipetted into sterile tubes and centrifugated at high speed, the cocci being resuspended in warm, sterile salt solution just prior to injection. The disadvantage of this procedure consisted in the varying dosage from day to day depending on the thoroughness with which the cocci were removed from the broth cultures.

Successful spinal puncture is not a difficult procedure in rabbits and dogs, providing large animals are employed and the back well arched. An ordinary Babcock spinal puncture needle cut off to a length of 4 cm. was used; this needle is of small caliber and when properly pointed serves well for the puncture of animals. In the first experiments each animal was etherized, but after experience had been gained it was proved possible to conduct the operation quickly and with a minimum of discomfort, rendering anesthesia unnecessary. In the operation each animal was placed on its right side and firmly held by an assistant with the back arched; the needle was entered slowly and cautiously in the lower lumbar region in the median line. Entrance into the subarachnoid space is felt by the hand of the operator in much the same way as experienced in conducting lumbar puncture on persons. On the withdrawal of the stylet, spinal fluid may not appear from rabbits and small dogs for a minute or more, owing to the slight pressure of the fluid under normal conditions and the length of needle to be traversed.

As preliminary experiments had shown that the subthecal injection of solutions of dyes in an amount of 0.5 c c in the lumbar region of rabbits is followed within 3 hours by diffusion along the entire length of the cord and base of the brain with slight diffusion over the cerebrum, all injections were made in the lower lumbar region instead of in the region of the occipito-atlantoid ligament. This is probably a somewhat easier route for puncture, but the animal is more likely to be injured. Experiments have shown that the amount of fluid injected subthecally in rabbits weighing from 1,500 to 3,000 gm. should not be more than 0.5-1.0 c c; even when these amounts are used a temporary paralysis of one or both hind legs may follow the operation. In dogs weighing 6 kilo or more, about 1 c c of fluid may be injected without harm, but it appeared advisable in our experiments never to inject more than 1.5 c c.

In all experiments careful records were kept of the temperature and clinical condition of each animal and changes in the total number of leukocytes in the blood; owing to the small size of the majority of rabbits, it was difficult to secure during life more than a drop or two of spinal fluid for cytologic and bacteriologic examinations, although as much as 0.5-1 c c may be obtained from large dogs.

EFFECT OF INTRAVENOUS INJECTION OF VIRULENT PNEUMOCOCCI IN NORMAL RABBITS

The intravenous injection of virulent pneumococci into a series of normal rabbits was never followed by the development of meningitis. The doses were so regulated according to body weight as to prolong the lives of many of the animals for at least three days. Necropsy examination was made immediately after death, but without exception it failed to disclose any macroscopic or bacteriologic evidence of invasion of the meninges or cerebrospinal fluid. Most interest centered in the type 2 strain because it produced a fatal spontaneous meningitis in a child. It was employed in some of these experiments in the first broth culture from fresh spinal fluid, but the results were negative and showed that an induced bacteremia was not followed by an invasion of the normal meninges and spinal fluid of rabbits as shown in the following protocol:

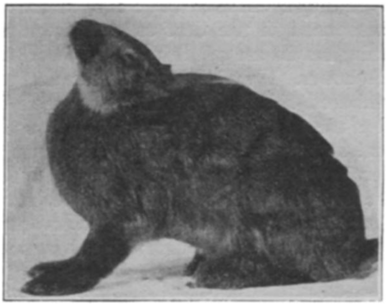

Fig. 1.—Opisthotonos in a rabbit 48 hours after the subthecal injection of 0.01 c c of type 2 pneumococci in salt solution.

A rabbit, weighing 2,000 gm., with a temperature of 38 C., with a leukocyte count of 10,200, received intravenously 0.5 c c of a suspension of type 2 pneumococcus secured by centrifuging 5 c c of an 18-hour broth culture and resuspending the cocci in 5 c c of salt solution. Twenty-four hours later the temperature was 40 C. and the leukocyte count was 20,600. The animal was very ill but presented no evidence of hypersensitiveness or increased reflexes. It died three days after the injection showing no evidence of involvement of the brain and cord. A culture of the heart's blood showed pneumococci; smears and cultures of the spinal fluid were sterile.

EFFECTS OF INTRAVENOUS INJECTION OF VIRULENT PNEUMOCOCCI IN NORMAL DOGS

Similar results were observed in normal dogs; the intravenous injection of large doses of pneumococci did not produce meningitis in any of our animals. A dog weighing 6,000 gm., with a rectal temperature of 36.5 C., and with a leukocyte count of 10,200, received an intravenous injection of 30 c c of a 24-hour broth culture of type 2 strain which killed mice weighing 17 to 20 gm. in 48 hours when given a dose of 0.0001 c c. Twelve hours later the temperature was 41 C. and the leukocytes numbered 21,900; the animal appeared sick and refused food. Twenty-four hours later the temperature was 39.5 C., the leukocytes numbered 20,200, and the animal was quite active and had a good appetite. Seventy-two hours later the temperature was 38 C. and the leukocytes numbered 15,000; the animal was active, had a good appetite and appeared normal. Necropsy revealed a clear, sterile spinal fluid of normal amount in the ventricles and subarachnoid space with no macroscopic changes in the spinal and cerebral meninges; smears and cultures of the heart's blood were sterile.

A dog weighing 7,000 gm., with a temperature of 37 C. and a leukocyte count of 10,000, received intravenously 30 c c of a 24-hour broth culture of type 3 strain of pneumococcus in the first culture from the cerebrospinal fluid of a fatal case of meningitis in an adult person; 0.001 c c killed mice weighing about 20 gm. in 24 hours. Twelve hours later the temperature was 39 C. and the leukocytes numbered 17,600; the animal refused food and appeared ill. Twenty-four hours later the temperature was 39.5 C. and the leukocytes numbered 18,000; the animal still appeared ill, was quiet and refused food. Forty-eight hours after injection the temperature was 38.5 C. and the leukocyte count was 16,400. The animal had a good appetite, moved about freely and actively and appeared to have fully recovered. Necropsy revealed no macroscopic changes in the abdominal and thoracic organs; the brain, cord and meninges appeared normal. The cerebrospinal fluid was clear, sterile and of normal volume; the heart's blood proved sterile.

These two protocols are typical of the results observed; it is possible that more virulent cultures injected intravenously would have produced meningitis as reported by Bull, but the three cultures of pneumococci employed in these experiments and in rather large doses (about 5 c c per kilo of weight of 24-hour blood-broth cultures) showed no evidence of localization of the diplococci in the tissue of the central nervous system.

EFFECT OF THE INTRAVENOUS INJECTION OF VIRULENT PNEUMOCOCCI FOLLOWED BY THE SUBTHECAL INJECTION OF STERILE SERUM OR BROTH

In experiments with rabbits and dogs, consisting in the intravenous injection of pneumococci followed immediately by the subthecal injection of sterile serum or broth, well developed meningeal irritation was produced in rabbits, but not in dogs, providing virulent cultures were employed. As shown by Flexner and Amoss,[6] the subthecal injection of sterile normal serum lowers the resistance of monkeys to the virus of acute anterior poliomyelitis, and Kolmer and Sekiguchi [7] have found that the subthecal injection of sterile normal serum into dogs facilitates the passage of antibodies from the blood into the cerebrospinal fluid.

---

[6] Jour. Exper. Med., 1917, 25, p. 525.

A rabbit weighing 2,000 gm., with a temperature of 38 C. and a leukocyte count of 12,000, received intravenously 0.5 c c of a 24-hour broth culture of the type 2 strain; one hour later 1 c c of sterile rabbit serum was injected subthecally in the lumbar region. Twenty-four hours later the temperature was 39 C. and the leukocytes numbered 16,000; the animal appeared ill, but showed evidence of meningitis. Forty-eight hours after the injections the temperature was 40 C. and the leukocyte count was 20,000; the animal was very sick, refusing food and presenting opisthotonos with greatly increased reflexes. On the third day death occurred. Necropsy revealed no gross lesions in the abdominal or thoracic organs except enlargement of the spleen. The cerebrospinal fluid was increased in amount and slightly cloudy; the spinal and cerebral meninges were hyperemic, especially the cervical portion of the cord. Hyperemia was more marked at the base than in the convex portions of the brain. Smears showed the presence of polymorphonuclear leukocytes and

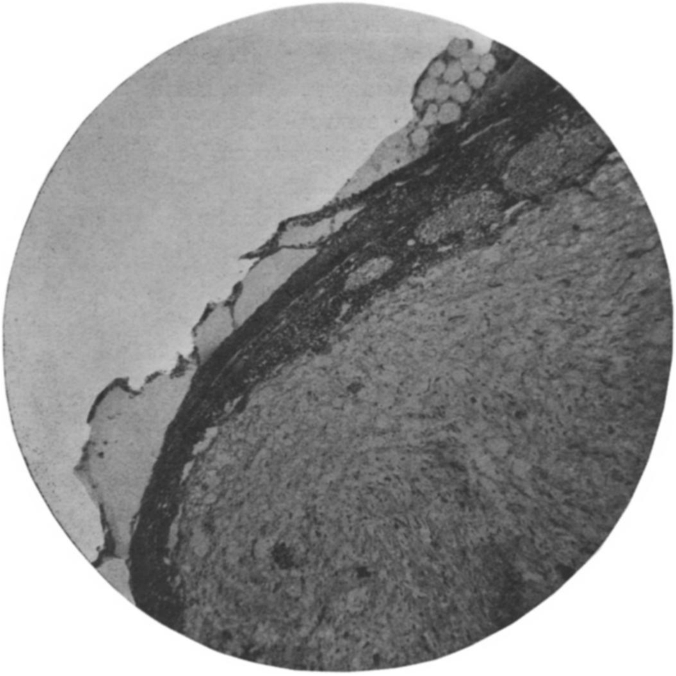

Fig. 2.—Section of the spinal cord in the dorsal region of a rabbit 48 hours after the subthecal injection in the lower lumbar region of type 1 pneumococci in salt solution; acute hyperemia and leukocytic infiltration of the meninges; × 70.

---

[7] Jour. of Immunology, 1918, 3, p. 101.

extracellular diplococci; cultures of the heart's blood and spinal fluid showed the presence of pneumococci. A control rabbit receiving a similar injection of pneumococci intravenously but without the subthecal injection of serum, presented absolutely no evidence of involvement of the meninges. A second control receiving 1.5 c c of the same serum subthecally in the lumbar region of the cord developed a temporary paralysis of both hind legs, but three days later was in normal condition and presented no discernible evidence of meningeal involvement.

The above protocol is illustrative of the results obtained in rabbits with pneumococci of such virulence that 0.0001 c c or less killed mice within 40 hours. Similar experiments with pneumococci of less virulence, requiring from 0.01 to 0.001 c c as the lethal dose for mice, invariably yielded negative results; that is, when injected intravenously with a subthecal injection of sterile rabbit serum the pneumococci showed no tendency for localization in the meninges. In dogs the results were generally negative, even with highly virulent cultures; the intravenous injection of doses of pneumococci as high as 0.5 c c per kilo of weight of 24-hour broth cultures followed one hour later by the subthecal injection of from 1 to 3 c c of sterile broth in the lumbar region of the cord, was not followed by any evidence of pneumococcus meningitis.

EFFECT OF THE SUBTHECAL INJECTION OF VIRULENT PNEUMOCOCCI IN THE LUMBAR REGION OF THE SPINAL CORD OF RABBITS

Well developed suppurative leptomeningitis was produced with regularity in rabbits following the subthecal injection of relatively large doses of virulent type 1 and type 2 pneumococci suspended in warm normal salt solution; the best results were observed with the type 2 strain, probably because of its greater virulence.

Immediately following the injections many of the animals showed temporary paralysis of one or both hind legs probably due to pressure. Within from 12 to 18 hours the temperature and number of leukocytes of the blood increased and the animals appeared sick, refusing to eat or to move about in their cages. Within 24 hours hypersensitiveness was generally apparent; many of the animals sat with their heads retracted in the position of opisthotonos and tapped the forehead or produced muscular tremors or convulsions along the vertebrae. Usually the animals remained in this condition, and it was accompanied with high fever, leukocytosis, loss in weight and increasing apathy until death occurred on from the second to the fifth day following the lumbar puncture. Spinal puncture on the second day usually showed an increased volume of cloudy spinal fluid, as several drops could be obtained, whereas in normal rabbits not more than a drop or two appeared at the end of the needle after an interval of a minute or more. Smears of the fluid showed large numbers of polymorphonuclear leukocytes with a few mononuclears and large numbers of diplococci, which were almost invariably extracellular.

At necropsy the abdominal and thoracic organs appeared normal except for acute hyperemia of the spleen, which was frequently noted; culture of the heart's blood invariably showed the presence of pneumococci. The meninges of the cord and usually of the brain, particularly at the base, showed well defined hyperemia with an increase of spinal fluid filling the sulci of the brain. The pia mater was usually dull and flakes of exudate composed of fibrin, polymorphonuclear leukocytes and extracellular pneumococci were found plastered to the pia of the cord and occasionally at the base of the brain; in a few instances portions of exudate were found over the convex portions of

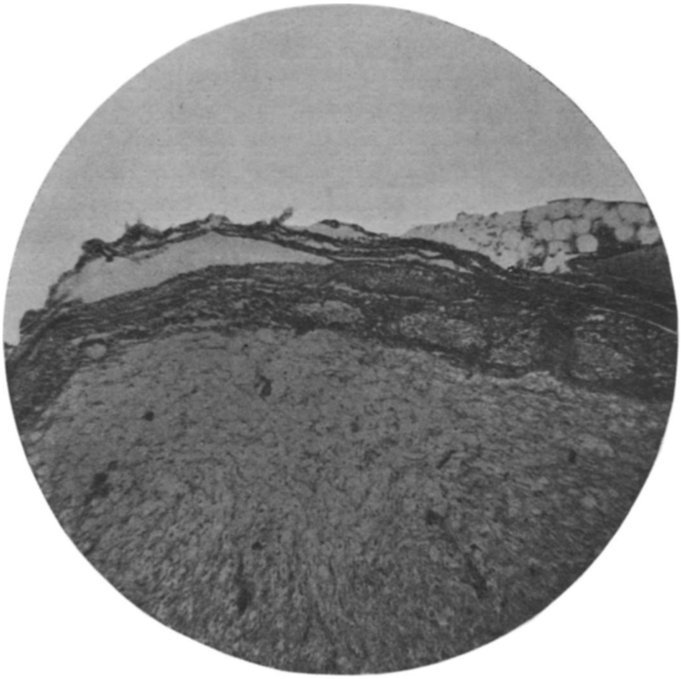

Fig. 3.—Section of the spinal cord in the dorsal region of a rabbit 48 hours after the subthecal injection in the lower lumbar region of type 2 pneumococci in salt solution; acute hyperemia and marked leukocytic infiltration of the meninges with extension in the cord; × 70.

the cerebrum. The spinal fluid was cloudy and contained large numbers of polymorphonuclear leukocytes and extracellular diplococci. Hyperemia and exudation were invariably most marked over the first 4 to 6 cm. of the cord and near the site of the lumbar puncture; in a few instances injury of the spinal cord was found, due to piercing with the spinal puncture needle. Not infrequently marked hyperemia and exudation were apparent in the cerebral portion of the cord, which was some distance from the site of injection of the pneumococci.

Histologic examination of the spinal cord and meninges at different levels showed well marked hyperemia and intense leukocytic infiltration of the meninges with slight edema of the substance of the cord adjacent to the pia mater and extension of the leukocytic infiltration along the perivascular spaces for some distance into the cord.

These changes were most marked in sections of the cord and meninges removed within 4 cm. or less of the site of injection; smaller, but well defined and similar, changes were frequently present in sections removed from the cervical portion of the cord and base of the brain. The following protocols of two experiments are illustrative of the results observed:

A rabbit weighing 2,000 gm. with a temperature of 38.5 C. and a leukocyte count of 11,200, received a subthecal injection in the lumbar region of 1 c c of a suspension of type 1 pneumococci in salt solution secured by centrifuging 5 c c of a 24-hour broth culture and resuspending the cocci in 5 c c of salt solution. Immediately after the injection the animal showed slight paralysis of the left hind leg. Twenty-four hours later the temperature was 41.5 C. and the leukocytes numbered 18,200. The animal presented increased reflexes and opisthotonos. On handling, generalized convulsion occurred. Necropsy showed no changes in the abdominal or thoracic organs; the cerebrospinal fluid was increased in amount, slightly cloudy and showed numerous polymorphonuclear leukocytes and extracellular diplococci. The meninges of the cord were hyperemic, particularly in the lumbar region near the site of injection. Small amounts of exudate were found along the cord and base of the brain; the meninges over the convex portion of the cerebrum showed hyperemia but no exudate. Cultures of the spinal fluid and heart's blood showed the presence of pneumococci in pure culture. Histologic examination of the cord and brain revealed marked hyperemia with infiltration of the meninges. There were moderate numbers of polymorphonuclear and mononuclear leukocytes. No changes in the substance of the brain and cord could be discovered.

A rabbit weighing 2,700 gm., with a temperature of 37.5 C. and a leukocyte count of 10,200, received a subthecal injection in the lumbar region of 0.5 c c of a suspension of type 2 pneumococci in physiologic salt solution recovered by centrifuging 3 c c of a broth culture. Six hours later the temperature was 40.5 C. and the leukocytes numbered 14,000. Twenty-four hours later the animal was hypersensitive and presented muscular weakness and beginning paralysis of both hind legs. The temperature rose to 41.5 C. and the leukocytes numbered 22,000. Forty-eight hours after the injection the animal was very ill with retention of urine, complete paralysis of the hind legs and marked opisthotonos. The temperature rose to 41 C. and the leukocytes numbered 12,000. It died about 60 hours after receiving the injection. At necropsy the abdominal and thoracic organs showed no gross changes except acute hyperemia of the spleen. There was an increase of cloudy spinal fluid in both lateral ventricles and the subarachnoid space with a patch over the convex portion. A smear of the spinal fluid showed pus cells and numerous pneumococci, mostly extracellular. Cultures of spinal fluid and heart's blood showed pneumococci in pure culture. Histologic examination of sections of the brain and spinal cord revealed an acute suppurative leptomeningitis with extension into the substance of the cord and brain.

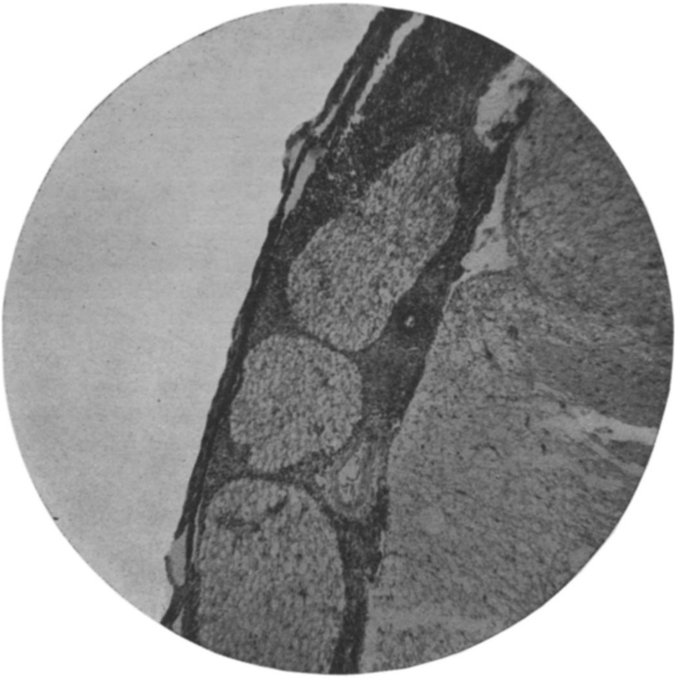

Fig. 4.—Section of the spinal cord in the dorsal region of a rabbit 48 hours after the subthecal injection in the lower lumbar region of type 2 pneumococci in salt solution; acute hyperemia and marked leukocytic infiltration of the meninges with extension in the cord; × 70.

EFFECT OF THE SUBTHECAL INJECTION OF VIRULENT PNEUMOCOCCI IN THE LUMBAR REGION OF THE SPINAL CORD OF DOGS

In similar experiments with dogs meningeal irritation and actual suppurative leptomeningitis were produced, but much less regularly and only with large doses of pneumococci. Acute hyperemia of the meninges of the cord, especially near the site of injection in the lumbar region extending to the base of the brain, with an increased volume of slightly cloudy spinal fluid in the ventricles and subarachanoid space, were the usual changes encountered. Flakes and patches of inflammatory exudate were rarely found, although histologic examination of the cord and meninges at different levels usually revealed some leukocytic infiltration confined to the meninges. Smears of the spinal fluid usually revealed a few polymorphonuclear and mononuclear leukocytes with scattered extracellular diplococci. Cultures of the spinal fluid were generally positive whereas cultures of the heart's blood were generally sterile.

The following protocols are illustrative of the results observed.

A dog weighing 6,500 gm., with a temperature of 38 C. and a leukocyte count of 14,200, was injected subthecally in the lumbar region with 2 c c of suspension of type 1 pneumococci in salt solution secured by centrifuging 10 c c of a 24-hour broth culture. This culture killed mice in 48 hours in a dose of 0.001 c c of 24-hour broth culture injected intraperitoneally. Forty-eight hours later the temperature rose to 39 C. and the leukocyte count was 19,200. The animal was very sick with ruffled hair, loss of appetite, some hypersensitiveness on handling, and he was too weak to stand. Spinal puncture removed about 0.5 c c of cloudy spinal fluid which revealed large numbers of pus cells and extracellular pneumococci; blood culture proved sterile. The animal died during the night. Necropsy disclosed a small patch of consolidation in the right lung with no other gross changes in the abdominal and thoracic viscera. On removal of the brain and cord there was found marked hyperemia of the meninges, particularly of the spinal portions, with marked increase of spinal fluid in the ventricles of the brain and subarachnoid space. Histologic examination revealed marked hyperemia of the meninges with leukocytic infiltration. Smears and cultures of spinal fluid showed polynuclear pus cells and extracellular diplococci; a culture of the heart's blood was sterile.

A dog weighing 10 kilo, with a temperature of 37.5 C. and a leukocyte count of 9,800, received a subthecal injection of 8 c.c. of a 24-hour broth culture of type 2 pneumococci of such virulence that 0.00001 c c killed mice of about 20 gm. in from 50 to 60 hours. Twelve hours later the temperature was 39 C. and the leukocytes numbered 16,800. The animal did not have the appearance of being ill. Twenty hours after the injection the animal died and necropsy showed a marked increase of cloudy spinal fluid in the ventricles and subarachnoid space with marked hyperemia of the meninges, especially in the lumbar region of the spinal cord near the site of injection of the pneumococci and in the cervical portion where petechial hemorrhages also were found in the pia mater. Smears of the spinal fluid showed large numbers of polymorphonuclear leukocytes and extracellular diplococci free of clumping; cultures of the spinal fluid and of the heart's blood were positive.

The following experiment yielded peculiar and interesting results as the animal appeared to recover from the immediate effects of a subthecal injection of pneumococci with the development of a well marked meningitis fourteen days later:

A dog weighing 7,000 gm., with a temperature of 38 C. and a leukocyte count of 10,200, was injected subthecally in the lumbar region with 2 c c of a suspension of type 1 pneumococci in physiological salt solution recovered by centrifuging 10 c c of a 24-hour broth culture. Twenty-four hours later the temperature was 38.5 C. and the leukocyte count was 22,400. During the following four days the dog appeared well but the number of leukocytes continued to be slightly increased—from 15,000 to 16,000. Fourteen days after the injection the animal became ill, showing these symptoms: ruffled hair, loss of appetite and beginning paralysis of the hind legs. The next day the animal was unable to stand, the hind legs were completely paralyzed. The bladder was also paralyzed leading to complete retention of urine; catheterizattion was necessary. The temperature was 39.5 C., and the leukocyte count was 15,400. The animal was killed with ether. Necropsy disclosed no gross changes in the abdominal and thoracic organs except an enlarged spleen. The meninges of the brain showed no changes except possibly an acute hyperemia, especially in the lumbar region. There was no visible exudate although the spinal fluid was slightly turbid and showed numerous pus cells and extracellular diplococci; culture of the spinal fluid and of the heart's blood showed the presence of pneumococci in pure cultures. Sections of the brain showed no histologic changes except slight hyperemia of the vessels of the pia mater; sections of the cord taken about 2 inches above the level of injection showed well marked hyperemia of the meninges and scattered areas of well marked infiltration with polynuclear and mononuclear leukocytes.

SUMMARY

These experiments show that pneumococcus cerebrospinal meningitis may be produced in rabbits by injecting virulent cultures directly into the subarachnoid space of the spinal cord. The reaction is much less marked in dogs, and usually takes the form of hyperemia without well defined suppurative changes, owing to the high resistance of this animal to the pneumococcus.

It would appear that large rabbits may be utilized in chemotherapeutic studies in pneumococcus meningitis because spinal puncture of this animal is not difficult; a definite series of pathologic changes in the spinal fluid and meninges with well defined symptoms of meningitis follows the subthecal injection of adequate doses of virulent pneumococci; and the supply of animals is unlimited. Furthermore, their carriage is generally semierect, as the majority sit up in their cages following spinal puncture. The disadvantages of using this animal are: its relatively small size; the difficulty in securing, during life, sufficient spinal fluid for examination, and the development of bacteremia, which probably is largely responsible for the early death of a majority of the animals. Monkeys are preferable because of their large size and erect carriage. Not infrequently, however, as in the rabbit, it is difficult to secure, during life, sufficient spinal fluid for examination. They also develop bacteremia, which probably is an important factor in shortening the duration of life and the period of observation. Furthermore, these animals may not be available in sufficient numbers for extensive chemotherapeutic experiments, or they may be too costly. Dogs are less satisfactory than monkeys and rabbits, owing to their high general resistance.

Intravenous injection of pneumococci alone into rabbits and dogs in our experiments has not resulted in the production of meningitis. Intravenous injection of pneumococci, followed by spinal puncture and the injection of sterile serum and broth, has usually elicited changes in the spinal meninges, chiefly in the nature of an acute hyperemia with slight or no leukocytic infiltration of the meninges and spinal fluid.

Subthecal injection of virulent pneumococci, however, produces a well marked suppurative leptomeningitis involving the entire spinal meninges, particularly in the immediate vicinity of the injection; the cerebral meninges, especially the basilar portions, are likewise involved, providing the animal survives at least 48 hours. What mechanism is involved in the production of acute hyperemia of the meninges or meningitis by the intravenous injection of pneumococci followed by the subthecal injection of the sterile broth or serum, is a matter of conjecture. That the subthecal injection of serum alone may produce hyperemia is a matter of direct observation as is also the fact that there is danger of direct injury to the meninges and cord which would permit the access of infected blood to the tissues and subarachnoid space. With small animals it is almost impossible to avoid producing considerable injury, and in some instance in our experiments the injections were made into the substance of the cord despite a considerable degree of care in conducting the spinal puncture.

CONCLUSIONS

The intravenous injection of virulent pneumococci, including two strains producing fatal meningitis in persons, into normal rabbits and dogs was not followed by the development of meningitis.

The intravenous injection of these virulent pneumococci into rabbits, followed by spinal puncture and the subthecal injection of sterile broth and serum, was usually followed by the production of acute meningeal congestion and occasionally by suppurative leptomeningitis. In dogs there was invariably acute congestion with no evidence of suppurative changes.

The subthecal injection of virulent pneumococci into the lumbar region of the spinal cord of rabbits is usually followed by the development of a fatal suppurative leptomeningitis accompanied by definite changes in the tissues and spinal fluid, fever, leukocytosis and clinical symptoms of meningitis, such as hyperesthesia, opisthotonos and convulsions. These changes appear to be sufficiently definite to permit the use of large rabbits for chemotherapeutic experiments in pneumococcus meningitis when they are infected by the direct subthecal injection of virulent pneumococci.

Spinal meningitis may be produced in dogs by the subthecal injection of large amounts of highly virulent pneumococci, but the results are more irregular than those observed in rabbits, and generalized suppurative changes occur only occasionally.